

Particle Film Affects Black Pecan Aphid (Homoptera: Aphididae) on Pecan

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ABSTRACT Three species of aphids attack pecan foliage, *Carya illinoensis* (Wang.) K. Koch, and cause economic damage. We tested a kaolin-based particle film against one of these aphid species, black pecan aphid, *Melanocallis caryaefoliae* (Davis). Effect of particle film on host selection, adult mortality, and production of nymphs by *M. caryaefoliae* was tested on seedling pecans in the laboratory. Fewer *M. caryaefoliae* adults selected treated foliage compared with untreated foliage. A higher percentage of adults that did select treated foliage were recovered from upper leaf surfaces compared with the percentage of adults recovered from upper leaf surfaces of untreated leaves. Observations with a microscope revealed an accumulation of particle film on aphid body parts, especially on tarsi, and strongly suggests that aphid mobility was restricted. Adult mortality was higher on treated foliage and led to an overall decrease in production of nymphs on those seedlings. In addition, we measured spectral properties of treated seedling pecan foliage. Light reflectance by treated foliage was increased and absorptance decreased compared with control foliage whereas transmittance of light through control and particle film-treated leaves was similar. We did not detect any phytotoxic effect on pecan due to application of particle film.

KEY WORDS *Melanocallis caryaefoliae*, Homoptera, Aphididae, particle film, kaolin, pecan

PECAN, *Carya illinoensis* (Wang.) K. Koch, foliage is attacked by three aphid species [black pecan aphid, *Melanocallis caryaefoliae* (Davis), blackmargined aphid, *Monellia caryella* (Fitch), and yellow pecan aphid, *Monelliopsis pecanis* Bissell] across the southeastern United States (Teddners 1978). Their feeding can cause a reduction of leaf chlorophyll and leaf area, a decrease in leaf net photosynthesis, depletion of carbohydrate reserves in stem tissue and defoliation (Teddners 1978, Teddners and Wood 1985, Wood and Teddners 1982, Wood et al. 1985). Aphids feeding on pecan also affect pecan yield, quality and return bloom (Dutcher et al. 1984, Wood et al. 1987). In addition, production of honeydew by aphids and its deposition on pecan foliage provides a substrate for growth of black sooty mold which reduces photosynthesis (Teddners and Smith 1976). In 1997, the damage to pecan and the cost of control of these aphids, in Georgia alone, was estimated at nearly \$8 million (Ellis and Dutcher 1999). As with many crops, conventional pest management in pecan orchards relies on chemical insecticides, but alternatives are needed.

A kaolin-based particle film has recently been proposed as a new management option for control of

certain arthropod and disease pests of agricultural crops (Glenn et al. 1999, Lapointe 2000, Puterka et al. 2000). Glenn et al. (1999) and Puterka et al. (2000) report on particle films for suppression of pear psylla, *Cacopsylla pyricola* Foerster, pear rust mite, *Eritrimerus pyri* (Nalepa), twospotted spider mite, *Tetranychus urticae* Koch, potato leafhopper, *Empoasca fabae* (Harris) and spirea aphid, *Aphis spiraeicola* Patch. An earlier study also showed that kaolin, when applied as a suspension to citrus, reduced colonization by spirea aphids (Bar-Joseph and Frenkel 1983). In addition, Lapointe (2000) showed that application of particle film to citrus foliage reduced feeding damage by adult citrus root weevils, *Diaprepes abbreviatus* (L.), and that oviposition by *D. abbreviatus* on the treated foliage was completely suppressed. Based on these studies, we hypothesized that particle film may provide an alternative to chemical control for *M. caryaefoliae* and other aphid pests of pecan.

Our objective was to use laboratory studies to examine effects of particle film on the most-economically important aphid species attacking pecan, *M. caryaefoliae*. We examined aspects of host selection, aphid mortality, and production of nymphs with respect to treated and untreated foliage. Because application of particle film does coat foliage with a white film and could interfere with host finding, we assessed the spectral properties of treated and untreated leaves of greenhouse-grown seedling pecans.

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Materials and Methods

All laboratory tests were done using *M. caryaefoliae* and seedling pecans germinated from open pollinated 'Curtis' nuts (Teddars et al. 1982). For each laboratory test, seedlings were from the same planting date and made uniform in size, structure and physiological age of foliage by pruning the terminal bud and leaving only three fully expanded primary leaves. An aphid colony was started from field-collected *M. caryaefoliae* and maintained on potted pecan seedlings at room temperature and a photoperiod of 14:10 (L:D) h. All adults of this species are alate and our rearing regime maintained aphids as asexual reproductives. In all tests, particle film (Surround, Engelhard Corporation, Iselin, NJ) (60 g/liter) was prepared in water using an adjuvant (M-03 Spreader/Sticker, Engelhard, Iselin, NJ) (1.2 ml/liter) and applied to foliage (using compressed air hand-sprayers) to near runoff, allowed to dry and then applied again. Foliage of control seedlings was treated similarly with water.

Choice Test. This test examined the effect of particle film on selection of pecan foliage by *M. caryaefoliae* adults. Four trials of this experiment were done on separate dates and each trial consisted of three treatments replicated four times. Seedling pecan foliage (upper and lower leaf surfaces) was treated with water, particle film, or adjuvant (1.2 ml/liter) without particle film. Seedlings (control, particle film and adjuvant) were randomly placed in a triangular arrangement equidistant from each other within a 122 by 61 by 61-cm cage at room temperature and a photoperiod of 14:10 (L:D) h. An untreated seedling, that served as a source of aphids, was prepared by placing the seedling into a cage containing trees infested with *M. caryaefoliae*. After 48 h, numbers of adult *M. caryaefoliae* on the source seedling were counted. Each aphid-source seedling was severed at the base (to induce aphid dispersal upon foliage dessication), mounted upright in a rubber stopper and centered within the triangular arrangement of test seedlings. Distance from the nearest leaf on the source seedling to the nearest leaf on any test seedling was never <10 cm. After 72 h, adult aphids on all test seedlings were counted. Of the total number of aphids responding to all treatments, percentage of *M. caryaefoliae* adults recovered from individual treatments was arcsine transformed (Zar 1996) and subjected to a one-way analysis of variance (ANOVA) (SAS Institute 1995). The Tukey-Kramer Honestly Significant Difference (HSD) test was used to separate means when a significant difference was detected ($P \leq 0.05$). Data are presented as untransformed means \pm SE. Numbers of aphids recovered from upper leaf surfaces compared with lower leaf surfaces were separately analyzed for each treatment using a paired *t*-test (SAS Institute 1995). Percentage of *M. caryaefoliae* adults recovered from upper leaf surfaces of each treatment was arcsine transformed (Zar 1996) and subjected to a one-way ANOVA (SAS Institute 1995). The HSD test was used to separate means when a significant difference was

detected ($P \leq 0.05$). Data are presented as untransformed means \pm SE.

No-Choice Test. Three trials of this experiment were done on separate dates in settings as previously described but only with control and particle film-treated seedlings. Each experimental unit consisted of two seedlings, of the same treatment, placed on opposite sides of a cage and equidistant from an aphid source seedling centered in the cage. The source seedling was prepared and cut as in the prior experiment. Each experimental unit was replicated four times on each of the three dates. After 72 h, numbers of aphids on test seedlings were counted and their location on upper or lower leaf surfaces recorded. Percentage of *M. caryaefoliae* adults recovered from control and treated trees was subjected to a one-way ANOVA (SAS Institute 1995). Numbers of aphids recovered from upper leaf surfaces versus numbers from lower leaf surfaces were separately analyzed for each treatment using a paired *t*-test (SAS Institute 1995). Percentage of *M. caryaefoliae* adults recovered from upper leaf surfaces of treated versus control seedlings was arcsine transformed (Zar 1996) and subjected to a one-way ANOVA (SAS Institute 1995). Data are presented as untransformed means \pm SE.

Adult Mortality and Production of Nymphs. Clip-Cage Test. The effect of particle film on *M. caryaefoliae* adult mortality and production of nymphs was tested in environmental chambers at $27 \pm 1^\circ\text{C}$ and a photoperiod of 14:10 (L:D) h. Thirty pecan seedlings, prepared as previously described, each had one of the three leaves randomly selected and treated with particle film and another leaf randomly selected and treated with water. Fourth-instar *M. caryaefoliae* were transferred, using a camel's-hair brush, from the aphid colony to the test seedlings and placed singly on the underside of the particle film leaf and on the control leaf. Fourth instars were confined to distal ends of leaves and lateral to the midrib using clip cages. Although similar to clip cages used by Tedders and Wood (1987), construction and materials differed. Our clip cages were made from 0.5-cm-long sections of acrylic tubing (United States Plastic Corporation, Lima, OH) with an inner diameter of 0.95 cm. Tines of a hair clip were flame heated and top and bottom pairs of tines melted into the outer wall of two separate sections of tubing. The spring-loaded hair clips provided a means for keeping the two sections of tubing appressed to the upper and lower leaf surfaces. For the one section of tubing that would house aphids, fine-mesh screen was glued to the end of the tubing section that did not contact the leaf surface. After 48 h each aphid was moved (to a new position on the same leaf) across the midrib and then moved daily, for 5 d, toward the petiole rotating across the midrib. Daily movement of aphids to new positions on the same leaf was necessary because of reduced fecundity of *M. caryaefoliae* when progeny remained clustered near the female (Kaakeh and Dutcher 1992). Immediately following removal of the aphid to a new position, the position previously occupied by

the aphid was cut away and nymphs counted. Mortality of adult aphids on control and particle film-treated leaves was analyzed using the Wilcoxon Signed Rank Test (SAS Institute 1995). Numbers of nymphs produced on control and particle film-treated leaves were analyzed using a paired *t*-test (SAS Institute 1995).

Whole-Plant Test. This test was done in three separate environmental chambers each at $27 \pm 1^\circ\text{C}$ and a photoperiod of 14:10 (L:D) h. We used a randomized complete block with four blocks and two treatments for each chamber. Experimental units were set up by placing a single control or particle film-treated seedling into a cylindrical cage ($h = 46$ cm, $r = 14$ cm) made of clear polyester (Mylar, United States Plastic, Lima, OH). White 'five-gallon' bucket lids (Letica, Rochester, MI) served as cage bottoms and tops with the bottom lid vented by four, equally spaced 5-cm-diameter openings around the periphery and the top lid vented by one 9-cm-diameter opening in the center. All vents were covered with a fine mesh screen. Three *M. caryaefoliae* fourth instars were placed, one per leaf, on the upper leaf surface of each leaf for both control and particle film seedlings. After 5 d (1 d to complete development and 4 d as an adult), interior surfaces of cages and the seedlings were examined for adults and nymphs. For each treatment we recorded percentage mortality of adults, location of surviving adults (upper or lower leaf surface), total nymphs produced by the three adults and location of nymphs (upper or lower leaf surface). Percentage mortality of adults was arcsine transformed (Zar 1996) and analyzed using ANOVA. Data for percentage mortality of adults is presented as untransformed means \pm SE. Nymphs produced between treatments was analyzed using ANOVA. Within treatments, location of adults and nymphs (upper versus lower leaf surfaces) was separately compared using a paired *t*-test. When data did not meet assumptions of normality for the *t*-test, the nonparametric Wilcoxon signed rank test was used (SAS Institute 1995).

Spectral Assessment. The influence of particle film on the spectral properties of pecan leaves was assessed using fully expanded, mature, simple leaves of five greenhouse-grown seedling pecans treated with particle film and five control seedlings. Treated and control trees were prepared as previously described except trees were not pruned. Spectral properties were assessed about 48 h after application using a portable spectroradiometer (LI-800, LI-COR, Lincoln, NE) coupled to an external integrating sphere (LI-1800-12S, LI-COR, Lincoln, NE). The apical-most mature leaf of each seedling was detached and immediately placed in the sphere for measurement. The proportion of light (from 360–850 nm) reflected, and transmitted, by leaves of control and treated seedlings was measured and recorded at 2-nm increments from 360 to 850 nm. The proportion of light absorbance by leaves was calculated as: $\text{Absorbance} = 1 - (\text{proportion reflected} + \text{proportion transmitted})$.

Results

Choice Test. Aphid source seedlings were infested with 285 ± 18 (mean \pm SE) adult aphids per seedling before severing at the base. The mean percentage of live, adult aphids recovered per cage at the end of the study was $71 \pm 3\%$. There was no significant interaction between trial date and treatment ($P = 0.5862$), therefore, data were combined. Of the adults that had responded to a treatment, a significantly lower percentage had selected particle film-treated trees compared with the percentage selecting control or adjuvant-treated trees ($F = 31.67$; $df = 2, 45$; $P < 0.0001$) (Fig. 1A). Within each treatment (control, adjuvant and particle film), significantly more aphids occurred on lower leaf surfaces than on upper leaf surfaces ($t = 8.82$; $df = 15$; $P < 0.0001$, $t = 12.09$; $df = 15$; $P < 0.0001$ and $t = 4.17$; $df = 15$; $P = 0.0008$, respectively) (Fig. 1B). However, between treatments, a higher percentage of aphids was recovered from the upper leaf surfaces of particle film-treated leaves than from upper surfaces of control or adjuvant-treated leaves ($F = 91.47$; $df = 2, 45$; $P < 0.0001$) (Fig. 1C).

No-choice Test. Aphid source seedlings used in this experiment were infested with 463 ± 36 (mean \pm SE) *M. caryaefoliae* adults per seedling. No significant interaction was detected between trial date and treatment ($P = 0.6990$), therefore, data were combined. When given no choice, a significantly higher percentage of *M. caryaefoliae* moved to control seedlings compared with treated seedlings ($F = 86.02$; $df = 1, 14$; $P < 0.0001$) (Fig. 2A). For both control and treated seedlings, more aphids were recovered from lower leaf surfaces compared with upper leaf surfaces ($t = 10.03$, $df = 15$, $P < 0.0001$ and $t = 2.39$, $df = 15$, $P = 0.03$, respectively) (Fig. 2B). But again, between treatments, a higher percentage of aphids was recovered from the upper surface of particle film-treated leaves than from the upper surface of control leaves ($F = 121.11$; $df = 1, 14$; $P < 0.0001$) (Fig. 2C).

Adult Mortality and Production of Nymphs. Clip-Cage Test. This experiment began with 30 replicated pairs of aphids but during daily transfer to new positions on leaves, some mortality occurred resulting in seven replicates being excluded from analyses. After 6 d, adult mortality on treated leaves was higher than on control leaves (signed rank statistic = -58.50 ; $df = 11$; $P = 0.002$) (Fig. 3A). Overall, more nymphs were produced by adults on control leaves than on treated leaves ($t = 2.71$, $df = 22$, $P = 0.013$) (Fig. 3B). When both aphids within a replicate did survive to the end of the study, as occurred in only four replicates (due to high mortality of aphids on treated leaves), nymphal production by survivors on control and treated leaves was 34.0 ± 4.6 and 32.5 ± 3.3 (mean \pm SE) nymphs per adult, respectively.

Whole-Plant Test. There was no significant interaction for environmental chamber \times treatment for adult mortality ($P = 0.2020$) or nymphs produced ($P = 0.2982$) between replications, therefore, data from separate environmental chambers were combined. When *M. caryaefoliae* adults were placed on control or

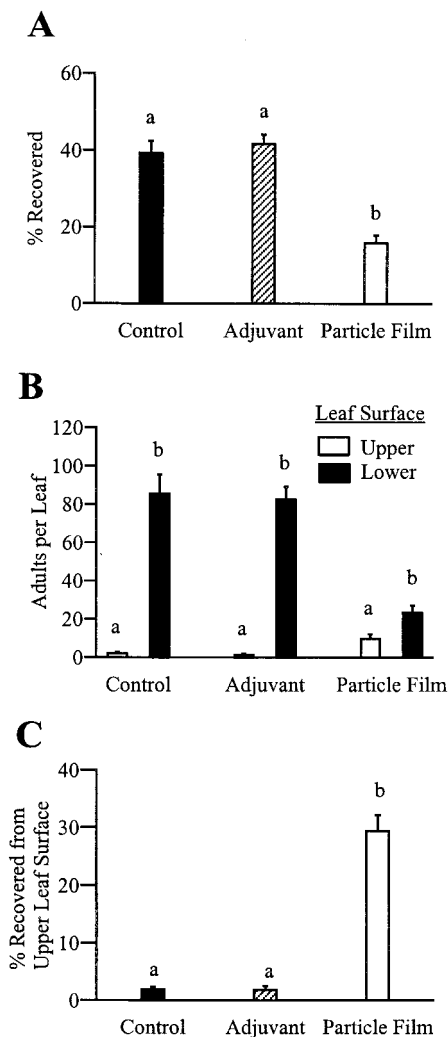


Fig. 1. Results of choice-test showing. (A) Percentage (mean \pm SE) of *M. caryaefoliae* adults that selected seedling pecan foliage treated with water (i.e., control), an adjuvant, or a kaolin-based particle film. (B) Number (mean \pm SE) of *M. caryaefoliae* recovered from upper versus lower leaf surfaces within each treatment. (C) Percentage (mean \pm SE) of *M. caryaefoliae* recovered from upper leaf surfaces of each treatment. Dissimilar letters above single columns or paired columns indicate significant difference ($P \leq 0.05$) between or within treatments, respectively.

treated seedlings in cages (but not confined to a limited portion of the seedling nor handled daily) adult mortality was significantly lower on control seedlings than on treated seedlings ($F = 5.16$; $df = 1, 23$; $P = 0.0492$) (Fig. 4A). The majority of surviving adults recovered from control leaves were located on lower versus upper leaf surfaces (signed-rank statistic = 18.00, $df = 11$, $P = 0.008$), whereas, recovery of surviving adults from upper versus lower surfaces of treated leaves was not statistically significant ($t = 1.91$, $df = 11$, $P = 0.0819$) (Fig. 4B). More nymphs were produced by adults on control seedlings than by adults

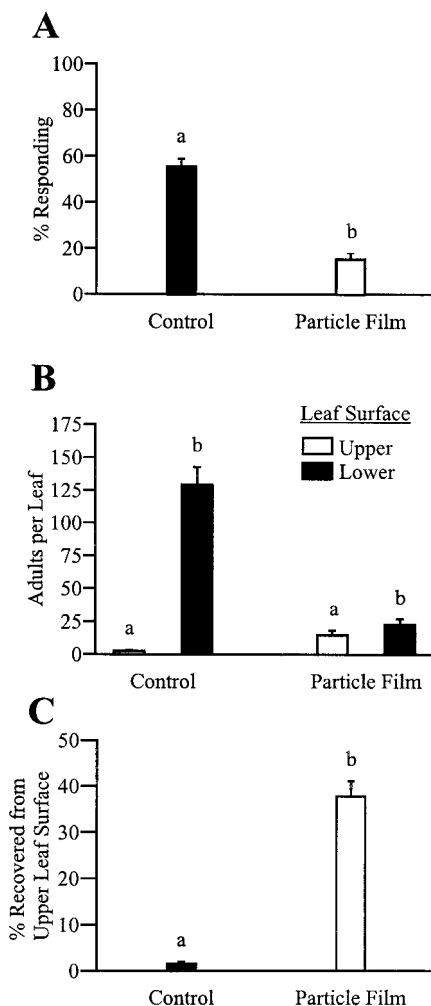


Fig. 2. Results of no-choice test showing. (A) Percentage (mean \pm SE) of *M. caryaefoliae* adults that selected seedling pecan foliage treated with water (i.e., control) and a kaolin-based particle film. (B) Number (mean \pm SE) of *M. caryaefoliae* recovered from upper versus lower leaf surfaces within each treatment. (C) Percentage (mean \pm SE) of *M. caryaefoliae* recovered from upper leaf surfaces of each treatment. Dissimilar letters above single columns or paired columns indicate significant difference ($P \leq 0.05$) between or within treatments, respectively.

on treated seedlings ($F = 9.66$; $df = 1, 23$; $P = 0.0126$) (Fig. 5A). In contrast with recovery of adults from upper versus lower leaf surfaces of control leaves, similar numbers of nymphs were recovered from upper and lower leaf surfaces of control leaves (signed-rank statistic = 19.5, $df = 11$, $P = 0.1338$). However, significantly more nymphs were recovered from upper versus lower leaf surfaces of treated leaves ($t = 5.53$, $df = 11$, $P = 0.0002$) (Fig. 5B).

Spectral Assessment. Spectral properties of treated leaves were affected by application of particle film. More light was reflected by pecan leaves treated with particle film than by control leaves at all measured

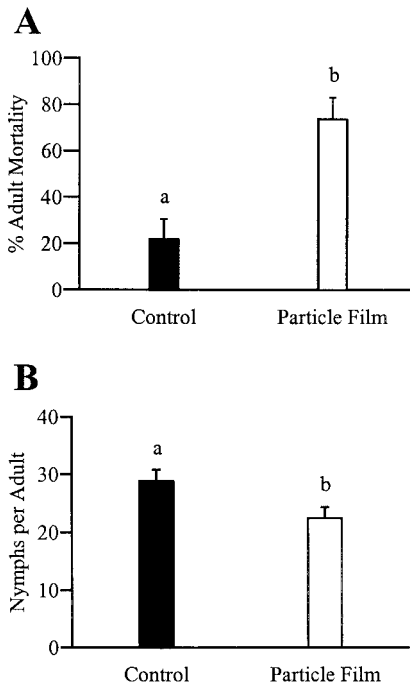


Fig. 3. (A) Percentage (mean \pm SE) mortality of *M. caryaefoliae* adults and (B) production of nymphs by adults in clip cages on control and particle film-treated pecan foliage. Dissimilar letters above columns indicate significant difference ($P \leq 0.05$).

wavelengths (Fig. 6A) whereas the reverse was determined for light absorptance (between 350 and 700 nm) (Fig. 6C). Light transmittance by leaves was similar for both treatments (Fig. 6B).

Discussion

Fewer *M. caryaefoliae* dispersed by flight to foliage treated with particle film than to foliage treated with water or the adjuvant. Many factors that affect host finding and acceptance by aphids have been studied and are reviewed by Klingauf (1987). Generally, aphids are attracted to blue-UV light from the sky when dispersing from host plants and then after a period of flight are especially attracted to foliage-like hues (500–580 nm) reflected from leaves (Klingauf 1987, Gibson and Rice 1989). In our study, lights were mounted on the exterior glass top of cages and *M. caryaefoliae* adults commonly flew near the cage top before settling on a new host. Pecan foliage treated with particle film was white and purified kaolin has been reported to have white brightness qualities $>85\%$ (Glenn et al. 1999). Bar-Joseph and Frenkel (1983) suggested that the whiteness imparted to foliage, from treating with kaolin suspensions, was responsible for decreased colonization of citrus by *A. spiraecola*. Klingauf (1987) states that perception of color by aphids does not allow for differentiation of host versus nonhost plants but rather plant shape, contrast and other optical characters (specifically the

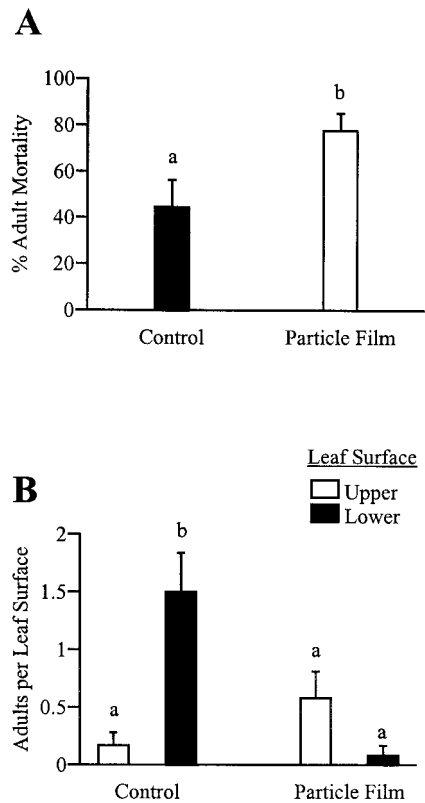


Fig. 4. (A) Percentage (mean \pm SE) mortality of *M. caryaefoliae* adults between treatments and (B) number (mean \pm SE) recovered from upper versus lower leaf surfaces within each treatment when three aphids were placed on caged plants. Dissimilar letters above single columns or paired columns indicate significant difference ($P \leq 0.05$) between or within treatments, respectively.

intensity of illumination) are important for inducing alightment during the attack flight. Given that more light was reflected from pecan foliage treated with particle film than from control foliage suggests that particle film interfered with visual host-finding cues used by *M. caryaefoliae*.

When aphids did alight on treated foliage, however, particle film may have served as a physical repellent or prevented tactile recognition of the hosts and stimulated some aphids to leave (Glenn et al. 1999). Glenn et al. (1999) observed that *C. pyricola* and *A. spiraecola* became coated with kaolin particles and that adult *C. pyricola* were preoccupied with removing the particles from body parts. In agreement, our microscope observations of aphids from treated foliage revealed an accumulation of particle film, especially on tarsi of aphids. In some instances, aphids repeatedly attempted to grasp the substrate by using a tarsus that was coated with particle film (T.E.C., unpublished data). Therefore, an accumulation of particle film on body parts of aphids in this study may explain why more aphids remained on upper leaf surfaces of treated foliage compared with control foliage in tests

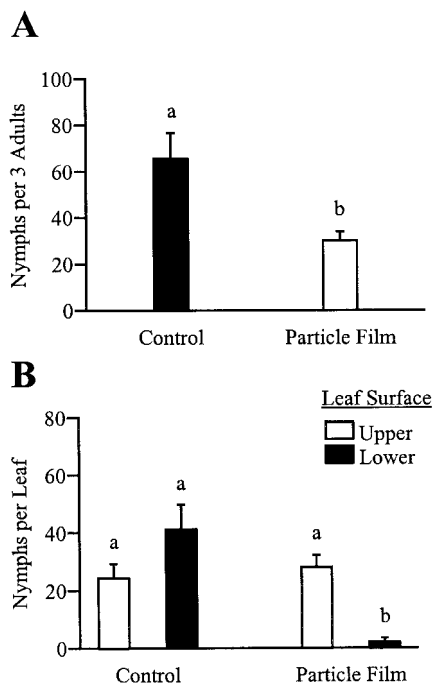


Fig. 5. (A) Production of nymphs (mean \pm SE) by three *M. caryaefoliae* placed on a caged plant and (B) location of recovered nymphs within each treatment. Dissimilar letters above single columns or paired columns indicate significant difference ($P \leq 0.05$) between or within treatments, respectively.

that did not confine aphids to a limited portion of the leaf. In a study using a population of *M. caryaefoliae* from a pecan orchard, Tedders (1978) stated that 74% of *M. caryaefoliae* (stages not reported) were found on the lower surfaces of pecan leaves during late summer and fall. In fact, in our study, direct placement of fourth-instar *M. caryaefoliae* onto upper leaf surfaces of control and particle film-treated foliage was done to facilitate transfer and prevent aphids from soon falling from leaves, as occurred with *A. spirea* adults placed on particle film-treated foliage (Glenn et al. 1999). This still resulted in a significant discrepancy in numbers of subsequent adults on upper leaf surfaces of control versus particle film-treated foliage. Even when adult aphids were not confined within clip-cages but rather placed in large cages containing either a particle film-treated or control seedling, more were recovered from upper surfaces of particle film-treated leaves than from control leaves.

Aphid mortality was higher for adult *M. caryaefoliae* on foliage treated with particle film than on control foliage and resulted in significantly fewer progeny being produced by adults on the treated foliage. Increased mortality may be caused by starvation due to the repellent nature of the particle film or altered insect behavior (e.g., increased grooming) in the presence of particle film as has been suggested by Glenn et al. (1999). However, when aphids survived being confined to a limited portion of a particle film-treated

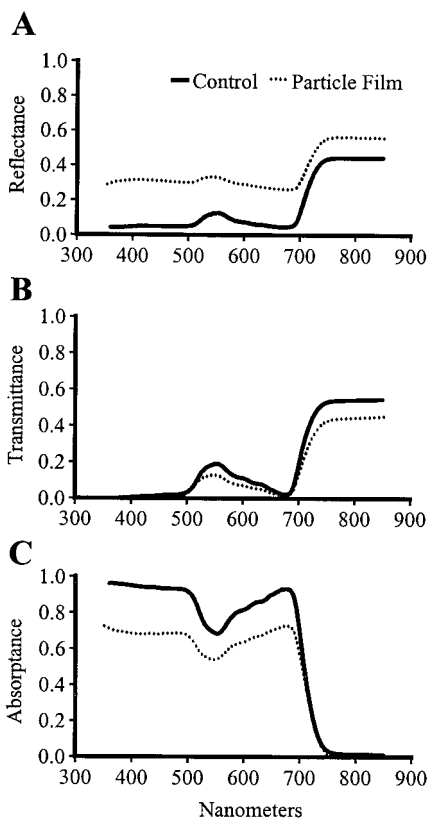


Fig. 6. Proportion of light that was (A) reflected, (B) transmitted, or (C) absorbed, by control and particle film-treated leaves of greenhouse-grown seedling pecans.

or control leaf, production of progeny was similar. It is possible that nonuniform application of particle film may have provided those aphids an opportunity to settle on areas of the leaf with less particle film.

This study has shown that kaolin-based particle film can affect *M. caryaefoliae* through interfering with host finding, decreasing production of progeny and increasing aphid mortality. Efficacy of particle film, however, is dependent on coverage and arthropod pests that favor a sessile behavior may settle on untreated areas and exploit a nonuniform coating. Testing particle film under orchard conditions will provide a better understanding of its potential for use against not only *M. caryaefoliae* but also against *M. caryella* and *M. pecanis* in commercial pecan production.

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